NeuroImage: Clinical 6 (2014) 463–474

Contents lists available at ScienceDirect

NeuroImage: Clinical

journal homepage: www.elsevier.com/locate/ynicl

Altered functional connectivity links in neuroleptic-naïve and neuroleptic-treated patients with schizophrenia, and their relation to symptoms including volition

Weidan Pu[a,b,1], Edmund T. Rolls[c,d,*,1], Shuixia Guo[e,1], Haihong Liu[a], Yun Yu[e], Zhimin Xue[a], Jianfeng Feng[f,g,*], Zhening Liu[a,*]

[a]Institute of Mental Health, Second Xiangya Hospital, Central South University, Changsha 410011, PR China
[b]Medical Psychological Institute, Second Xiangya Hospital, Central South University, Changsha 410011, PR China
[c]Oxford Centre for Computational Neuroscience, Oxford, UK
[d]Dept of Computer Science, University of Warwick, Coventry CV4 7AL, UK
[e]College of Mathematics and Computer Science, Key Laboratory of High Performance Computing and Stochastic Information Processing, Ministry of Education of China, Hunan Normal University, Changsha, Hunan 410081, PR China
[f]Centre for Computational Systems Biology, School of Mathematical Sciences, Fudan University, Shanghai 200433, PR China
[g]Department of Computer Science, University of Warwick, Coventry CV4 7AL, UK

ARTICLE INFO

Article history:
Received 10 June 2014
Received in revised form 1 October 2014
Accepted 11 October 2014
Available online 16 October 2014

Keywords:
Schizophrenia
Volition
Functional connectivity
Precuneus
Negative symptoms
Orbitofrontal cortex

ABSTRACT

In order to analyze functional connectivity in untreated and treated patients with schizophrenia, resting-state fMRI data were obtained for whole-brain functional connectivity analysis from 22 first-episode neuroleptic-naïve schizophrenia (NNS), 61 first-episode neuroleptic-treated schizophrenia (NTS) patients, and 60 healthy controls (HC). Reductions were found in untreated and treated patients in the functional connectivity between the posterior cingulate gyrus and precuneus, and this was correlated with the reduction in volition from the Positive and Negative Symptoms Scale (PANSS), that is in the willful initiation, sustenance, and control of thoughts, behavior, movements, and speech, and with the general and negative symptoms. In addition in both patient groups interhemispheric functional connectivity was weaker between the orbitofrontal cortex, amygdala and temporal pole. These functional connectivity changes and the related symptoms were not treated by the neuroleptics. Differences between the patient groups were that there were more strong functional connectivity links in the NNS patients (including in hippocampal, frontal, and striatal circuits) than in the NTS patients. These findings with a whole brain analysis in untreated and treated patients with schizophrenia provide evidence on some of the brain regions implicated in the volitional, other general, and negative symptoms, of schizophrenia that are not treated by neuroleptics so have implications for the development of other treatments; and provide evidence on some brain systems in which neuroleptics do alter the functional connectivity.

1. Introduction

Schizophrenia is a disorder often characterized by positive, cognitive, and negative symptoms (Mueser and McGurk, 2004), which may have different neurobiological bases (Rolls, 2012; Rolls et al., 2008). The positive (psychotic or thought disorder) symptoms may include hallucinations, delusions, and paranoia. The cognitive symptoms may include a failure to maintain attention, and deficits in short-term memory which is required to maintain attention. The negative symptoms may include reduced emotion and motivation, including reduced hedonia. The neuroleptic drugs, which include the phenothiazines such as chlorpromazine and the second generation drugs such as clozapine and olanzapine, treat mainly the positive symptoms. There is little evidence that treatment with neuroleptics substantially improves functional recovery (measured for example by whether the patient is in employment) for most people with schizophrenia (Bowie et al., 2006; Insel, 2010; Milev et al., 2005). This may be partly because neuroleptic drugs are not very effective in improving the cognitive and negative symptoms of schizophrenia (MacDonald and Schulz, 2009). In this study, we aimed at identifying neurophysiological deficits in schizophrenia which are resistant to current anti-psychotic drugs. Identifying these key deficits which are present in schizophrenia, and which remain untreated by neuroleptics, is the fundamental approach that we take in this paper, to help provide insights into possible targets for new therapy for schizophrenia, which may help towards a better functional recovery than is obtained currently with neuroleptic drugs.

* Corresponding authors.
E-mail address: Edmund.Rolls@oxcns.org (E.T. Rolls), zningl@163.com (Z. Liu).
[1] WD Pu, ET Rolls and SX Guo contributed equally to this work.

http://dx.doi.org/10.1016/j.nicl.2014.10.004

The etiology and neuropathophysiological mechanism of this debilitating and severe disorder still remain unclear. Over the last two decades, much neurophysiological evidence for structural and functional deficits in the brain in schizophrenia has been developed into a disconnectivity hypothesis (Frith, 1995). More direct evidence for the neurophysiological disconnectivity hypothesis comes mainly from functional Magnetic Resonance Imaging (fMRI) studies, particularly resting-state fMRI studies (Khamsi, 2012; Smith, 2012), which have shown widespread functional disconnectivity in distributed brain networks in schizophrenia. However, these studies have so far not revealed a consistent pattern (Bluhm et al., 2007; Meyer-Lindenberg et al., 2005; Whitfield-Gabrieli et al., 2009; Zhou et al., 2007).

The reason for the inconsistency of the functional disconnectivity in schizophrenia still remains unclear. It has been suggested that this may be predominantly accounted for by medication effects and methodological differences in functional connectivity (FC) analysis (Achard and Bullmore, 2007; Honey et al., 2003; Pettersson-Yeo et al., 2011; Stephan et al., 2009; Tao et al., 2013). There has been direct evidence that dopamine receptor antagonists can alter FC and brain network parameters (Achard and Bullmore, 2007; Honey et al., 2003), suggesting that antipsychotics may be a major confounding factor in the FC analysis in schizophrenia patients. Thus, patterns of functional disconnectivity in neuroleptic-naïve schizophrenia (NNS) and neuroleptic-treated schizophrenia (NTS) patients might be in part different due to the neuroleptic treatment, but also partly similar owing to the limited efficiency of current antipsychotics in treating all symptoms of schizophrenia (Bowie et al., 2006; Insel, 2010; Milev et al., 2005). One of the aims of the current investigation was therefore to examine the common and the different features of brain functional disconnectivity in untreated (NNS) and neuroleptic treated (NTS) patients with schizophrenia, and this is one of the first studies to perform this.

2. Methods

2.1. Participants

The participants were 22 first-episode neuroleptic-naïve schizophrenic (NNS) patients and 61 first-episode neuroleptic-treated schizophrenia (NTS) patients recruited from the Department of Psychiatry of the Second Xiangya Hospital of Central South University, Changsha, China. All the patients met the DSM-IV diagnostic criteria for schizophrenia, Patient version (SCID-I/P) (First et al., 1996) (inclusion criteria and clinical measures for the patients are provided in the Appendix). The patient groups were not selected to be different, with the NNS patients recruited for the study in the outpatient department before they were administered drugs. The symptoms of the patients were evaluated using the Positive and Negative Symptoms Scale (PANSS) (Kay et al., 1987) by three psychiatrists in our research team. Sixty healthy controls (HC) were recruited from the communities of Changsha, China. The inclusion and exclusion criteria for HC were the same as those for the schizophrenic patients other than the DSM-IV diagnostic criteria. Individuals with a family history of psychiatric illness among their first-degree relatives were excluded from the HC group.

All participants gave their written informed consent to participate in the study after the risks and benefits were discussed in detail. The study was approved by the ethics committee of the Second Xiangya Hospital, Central South University.

2.2. Construction and comparison of the whole-brain functional connectivity network

After the resting-state fMRI data preprocessing (the fMRI data acquisition and preprocessing are described in the Appendix), the whole brain was parcellated into 90 regions of interest (ROIs) according to the automated anatomical labeling (AAL) atlas (Tzourio-Mazoyer et al., 2002) (45 regions in each hemisphere, given in Table S1 in the Appendix), and the time series were extracted in each ROI by averaging the signals of all voxels within that region, and then linearly regressing out the influence of head motion and global signals. A correlation matrix for the functional connectivities was obtained for each subject. We calculated partial correlation coefficients because these remove the effect of any common input from all other brain regions. By calculating partial correlation coefficients between all pairs of ROIs, with all the remaining ROIs being controlling variables, a 90×90 correlation matrix was obtained for each subject. [In more detail, we used a triplets-ROI based partial-correlation approach to remove the mediation from third-party regions. For an arbitrary pair of regions i and j, its partial correlation is calculated multiple times, each time with one third party region k ($k = 1, 2, \ldots, 90$, $k \neq i$ and $k \neq j$) being controlled (i.e. the mediation from region k is removed). We call these 3 regions i, j and k a triplet in this case. Since there are altogether 90 brain regions, there will be 88 third-party mediators for i and j (i.e. 88 triplets), and thus 88 partial-correlation coefficients will be obtained for region pair i and j. We then pick the smallest one (in amplitude) as the partial-correlation coefficient between i and j (Guo et al., 2014; Tao et al., 2013), indicating that the largest influence among all third-party mediators is removed.] This matrix was binarized to facilitate further statistical analyses by producing a sparse connectivity matrix by setting to 1 all links in the matrix with $p < 0.01$, and to 0 otherwise. The sparsity values of the HC, NTS and NNS matrices produced by the use of the $p < 0.01$ criterion were 2.15%, 2.27%, 2.17% respectively. The use of this criterion of $p < 0.01$ can be supported in part because these sparsities are close to those of the sparsity of brain network connectivity as measured with diffusion tensor imaging (Hagmann et al., 2008). The use of this $p < 0.01$ criterion is also justified by our prior work, which shows that the links that are significantly different between patient groups identified with this approach are robust to variation of the $p < 0.01$ criterion within a range of 0.01–0.1 (Ji et al., 2014). Further discussion of the approach is provided elsewhere (Cheng et al., 2012).

2.3. Measures of functional connectivity effects

We used a risk difference approach, widely used in medical statistics (Laupacis et al., 1988; Warn et al., 2002), to test whether the binarized connectivity matrices were different between the patient and control groups. The effect associated with a particular link can be calculated from the following (risk difference) score (Tao et al., 2013):

$$S^{link} = \frac{L_p^{link}}{N_p^{link}} - \frac{L_h^{link}}{N_h^{link}}$$

where S^{link} is the score for a particular link, L_h^{link} is the number of links with a value of 1 in the population of healthy controls, N_h^{link} is the total number of healthy controls, L_p^{link} is the number of links with a value of 1 in the population of schizophrenics, and N_p^{link} is the total number of patients. A risk difference of 0 indicates that the link is equally likely to occur in both groups, a risk difference score greater than 0 indicates that the link is more likely to occur (i.e. to be 1) in the patient group than in the control group, and a score less than 0 indicates that the link is less likely to occur in the patient group. In order to obtain the statistical significance of the score S^{link} for a particular link, a permutation test was carried out (in which scores were created just on the assumption that the patients and controls were randomly allocated to a group).

In brief the rationale behind our approach for establishing functional connectivity changes is to measure the risk difference score for each link, and to test (using a permutation test) for each patient group which links have significantly different risk difference scores from the control group. We were able to validate the main findings obtained with this risk difference statistical approach by application of a false discovery rate (FDR) procedure to the whole brain 90×90 matrix of functional connectivity correlation values; and by application of a whole brain FDR procedure to identify link differences based on Fourier analyses of the time series of the two connected brain regions, as described in

the Results section. The risk difference measure was selected for the analysis described here because patients who have not been treated with neuroleptic drugs are rare, and although we were able to gather 21 NNS patients for this group, we wished to use a measure that was potentially sensitive to differences when the group size is not very large. Having selected this measure, all the main results as part of the statistical approach were then validated by use of an FDR approach, as described in the "Further analyses" section.

3. Results

3.1. Participant characteristics

The participant characteristics for the three groups are shown in Table 1. There were no significant differences as to the sex, and years of education between the three groups. The mean age of the groups was similar (HC = 27.2 years, NN = 24.8, NTS = 22.9 years) [though the difference was significant ($F = 5.176$, $p = 0.007$), with a post-hoc test showing that the NTS group was significantly lower in age than the HC group only (LSD − $t = 4.30$, $p = 0.002$)]. Illness course also showed no significant differences between the two patient groups. The sum scores for the different parts of the PANSS (Kay et al., 1987) were similar for the two patient groups, with values provided in the Table 1. Significant differences between the two patient groups in 10 subscale scores of the PANSS (Kay et al., 1987) were present, including delusion, hostility, stereotyped thinking, anxiety, tension, mannerisms and posturing, uncooperativeness, poor impulse control and preoccupation (see details in Table S2). Thus, consistent with previous studies (Kane, 1999; Rosenheck et al., 1999; Smith et al., 1996), some of the positive symptoms were among those treated by the neuroleptics, and many of the cognitive and negative symptoms appeared to be treated less efficiently by the neuroleptic drugs.

3.2. Comparisons of functional connectivity maps between the three groups

Using permutation tests on the risk difference scores, 20 significantly altered links were identified in the NNS group and 19 in the NTS group, compared to the HC group (Fig. 1 and Table 2).

First the four altered functional connectivity links present in both NNS and NTS patients are considered. They are marked with rectangles in Figs. 1 and 2. We note that negative scores reflect a weaker functional connectivity link, and positive scores a stronger link, in a patient group relative to controls, as the score reflects patient group − healthy controls. Links with negative scores (i.e. weaker functional connectivities in patients relative to controls) are shown in blue in Fig. 1, and stronger links in red.

The greatest reduction in functional connectivity in both NNS and NTS patients compared with HC is that between the posterior cingulate gyrus (PCG) and precuneus (PCUN) in the left hemisphere ($s = -0.19$ with $p = 0.02$ for NNS and $s = -0.24$ with $p = 0.002$ for NTS). Since both the PCG and PCUN are large in the human brain, to examine better the similarities of the changes in both patient groups, source location analyses for the altered voxels in these two regions were performed in NNS and NTS respectively (calculated as described in the Appendix). From Fig. 3 (panel B and C), it can be seen that the locations of the altered voxels in the PCG and PCUN in the NNS patients were similar to those in the NTS patients.

The correlation analyses with the symptoms measured in the PANSS across all patients showed that the change in the PCG.L–PCUN.L link was significantly correlated with the disturbance of volition ($r = 0.45$, $p = 0.002$) (Table 3), as well as with some of the other general symptoms within the PANSS, and the PANSS general and negative symptom subtotal scores. [Since we were specifically interested in the common altered links in the two patient groups, to test the hypothesis that the common functional alterations in the two patient groups are correlated with cognitive and negative symptoms, correlation analyses were only performed between the 4 common altered links in both groups of patients and the clinical measures in the patients. This keeps the number of tests performed down to reasonable numbers, but as a precaution only effects that were significant at $p < 0.05$ with FDR correction are shown in Table 3. These are canonical correlations between the symptoms and the Fourier link coefficients based on the time series of the two connected brain regions, as described in the 'Further analyses' section.]

Three interhemispheric functional connections were also reduced in both patient groups: those between the middle temporal pole (TPOmid) ($s = -0.17$ with $p = 0.03$ for NNS and $s = -0.28$ with $p = 0.00$ for NTS), the superior orbitofrontal cortex (ORBsup) ($s = -0.19$ with $p = 0.03$ for NNS and $s = -0.16$ with $p = 0.009$ for NTS), and the amygdala (AMYG) ($s = -0.17$ with $p = 0.02$ for NNS and $s = 0.21$ with $p = 0.001$ for NTS) (Fig. 2), with additional statistical analyses confirming the significant reductions in the strength of these links after whole brain correction with FDR provided in the 'Further analyses' section.

As an additional check that these four links were all reduced in both patient groups, a direct comparison of the functional maps between the two patient groups showed no significant differences between these common reduced functional connections, confirming that the weakened PCG–PCUN link and three interhemispheric links (bilateral ORBsup, AMYG and TPOmid) were commonly altered in both untreated and treated patients ($p > 0.05$, Table 2 and Fig. S2).

The functional connectivity links that are different between the two patents groups are now considered. Fig. 2 shows the different patterns of altered functional connectivity in the NNS and NTS patients. [The resting state networks (RSN) shown on the right of Fig. 2 are based on the constructed six-community structure for the whole brain from healthy subjects shown in Fig. S1 in the Appendix, and was constructed using data from more than 400 healthy brains (Tao et al., 2013). These six communities have a clear biological significance which can be classified as the default mode network (DMN) (RNS1), the attention network (RSN2), the visual system (RSN3), the auditory system (RSN4), the sensory-motor areas (RSN5), and the sub-cortical network (RSN6).] One difference evident in Figs. 1 and 2 is that there were more strengthened functional connections in NNS patients relative to the controls, whereas there were more weakened connections in NTS patients

Table 1
Sociodemographic and clinical variables of healthy controls, neuroleptic-naïve and neuroleptic-treated schizophrenia patients.

Variables	HC (n = 60)	NNS (n = 22)	NTS (n = 61)	Statistical test	
				F/t / χ^2	p
Sex, No. M/F	35/25	14/8	35/26	.27	.87
Age (SD), years	27.2 (6.6)	24.8 (8.6)	22.9 (7.5)	5.18	.007
Education (SD), years	13.5 (3.2)	12.6 (3.2)	13.5 (2.6)	1.83	.16
Course (SD), months	–	12.3 (16.0)	18.1 (16.1)	1.4	.16
PANSS positive sum score (SD)	–	22.5 (6.2)	19.0 (6.2)	−1.89	.06
PANSS negative sum score (SD)	–	22.7 (10.3)	21.2 (6.7)	−.63	.53
PANSS general psychiatric sum score (SD)		42.5 (14.9)	38.2 (10.5)	−1.23	.23
PANSS sum score (SD)	–	92.3 (31.8)	85.1 (20.3)	−1.16	.25

Note: HC, healthy controls; NNS, neuroleptic-naïve schizophrenia; NTS, neuroleptic-treated schizophrenia; PANSS, positive and negative syndrome scale; SD, standard deviation.

Table 2
Functional connectivity links that are different relative to controls in neuroleptic-treated (NTS) and neuroleptic-naïve (NNS) first-episode schizophrenic patients.

Discrepant functional links in NTS patients				Discrepant functional links in NNS patients			
Links	Score	p-Value	Corrln NTS-HC	Links	Score	p-Value	Corrln NNS-HC
Ins.R–PUT.R	−0.320	.000	−0.058	+HIP.L–PHG.R	0.358	.000	0.072
TPOmid.L–TPOmid.R	−0.276	.000	−0.145	ROL.R–STG.R	0.303	.001	0.122
IFGtriang.L– IFGtriang.R	−0.242	.002	−0.061	-ROL.R–INS.R	−0.270	.007	−0.078
PCG.L–PCUN.L	−0.239	.002	−0.063	MOG.L–IOG.L	0.265	0.01	0.073
AMYG.L–AMYG.R	−0.211	.001	−0.119	+IFGoperc.R–SMG.R	0.265	.006	0.053
PreCG.L–PoCG.L	−0.207	.008	−0.058	SFGdor.L–MFG.L	0.242	.005	0.119
+PreCG.L–PreCG.R	−0.194	.003	−0.071	ROL.L–INS.L	−0.242	.006	−0.073
-PreCG.L–IFGoperc.L	0.191	.020	0.077	+SFGmed.L–ANG.L	0.239	.018	0.048
SFGmed.L–ANG.L	−0.189	.011	−0.072	MFG.R–IPL.R	−0.217	.021	−0.125
+IPL.L–IPL.R	−0.162	.006	−0.047	PreCG.R–SMA.R	0.211	.018	0.052
ORBsup.L–ORBsup.R	−0.160	.009	−0.070	+SFGdor.R–MFG.R	0.209	.009	0.105
SOG.L–MOG.L	0.152	.015	0.079	+PAL.L–THA.L	0.208	.022	0.032
SOG.L–SOG.R	−0.146	.005	−0.069	ROL.L–ROL.R	−0.206	.002	−0.072
PAL.L–PAL.R	−0.144	.028	−0.062	+IFGtriang.L–ORBinf.L	0.194	.029	0.064
SPG.R–IPL.R	−0.143	.028	−0.024	ORBsup.L–ORBsup.R	−0.192	.029	−0.078
ORBsup.L–ORBmid.L	0.136	.020	0.076	PCG.L–PCUN.L	−0.191	.022	−0.070
HES.L–STG.L	0.134	.007	0.041	AMYG.L–AMYG.R	−0.173	.016	−0.115
HIP.L–HIP.R	−0.129	.024	−0.091	+PUT.R–PAL.R	0.171	.006	0.043
PHG.L–PHG.R	−0.114	.002	−0.063	TPOmid.L–TPOmid.R	−0.168	.030	−0.114
				IFGoperc.L–IFGtriang.L	−0.120	.002	−0.091

Note: the positive scores reflect a greater proportion of patients than healthy controls with functional connectivity more significant than the binarization threshold ($p = 0.01$ for the partial correlation) for that link than controls, and negative scores a smaller proportion of patients with functional connectivity more significant than the binarization threshold than healthy controls. The scores were calculated with the equation described in the Methods section. The significance level for the risk difference p-value was set at $p < 0.03$. NTS: neuroleptic-treated schizophrenia; NNS, neuroleptic-naïve schizophrenia The names and abbreviations of the regions of interest are shown in Table S2.
− This indicates that there were fewer ($p < 0.05$) binarized links (with the binarization at $p < 0.01$) in the NNS than in the NTS group as shown by a permutation test.
+ This indicates that there were more ($p < 0.05$) binarized links in the NNS than in the NTS group as shown by a permutation test.
NTS-HC shows the value of the functional connectivity (measured by the partial correlation coefficients obtained from the resting state fMRI analysis) in the NTS group minus that in the healthy control group. A negative value thus shows a weaker functional connectivity in the NTS group than in the healthy control group. NNS-HC: the same for the functional connectivity in the NNS group minus that in the healthy control group.To help with the interpretation, all the functional connectivity values for the links in the table were positive, and the mean value of the functional connectivity for the healthy controls across these links in Table 2 was 0.31 (which was the mean of the partial correlation coefficients). Further, a negative risk difference score for a patient group − HC reflects a lower positive functional connectivity correlation value for a link in a patient group than in the HC group.

relative to controls. The statistically significant differences between the NNS and the NTS groups are shown by + or − prescripts in Table 2, and the differences are indicated in Fig. S2.

The altered functional connectivity in the NNS group was mainly in fronto-parietal circuits and subcortical circuits (see Fig. 2A). The abnormal fronto-parietal circuits were composed of three different neural pathways, including a circuit (i.e. a set of altered connected functional links) involving the dorsal superior frontal gyrus (SFGdor), middle frontal gyrus (MFG), and inferior parietal lobule (IPL); a circuit involving the inferior obitofrontal cortex (ORBinf), opercular inferior frontal gyrus (IFGoper), triangular inferior frontal gyrus (IFGtriang) and supramarginal gyrus (SMG); and a circuit involving the medial superior frontal gyrus (SFGmed) and angular gyrus (ANG) (see Fig. 2A). The subcortical circuits included a striato-thalamic circuit involving the putamen (PUT), pallidus (PAL) and thalamus (THA); and a hippocampo-parahippocampal circuit. In addition, an insula-rolandic-superior temporal circuit in the auditory network, a circuit in the sensorimotor network involving precentral gyrus (PreCG) and supplementary motor area (SMG), and a circuit in the visual network involving middle occipital gyrus (MOG) and inferior occipital gyrus (IOG) were also identified as discrepant in the NNS patients (see Fig. 2A).

The altered functional connectivity in the NTS group was mainly in a prefronto-sensorimotor circuit involving IFGoper, PreCG and postcentral gyrus (PoCG); and a sriato-insular circuit involving PUT and insula (INS) (see Fig. 2B). Further, it is noteworthy that the abnormal interhemispheric connectivity was more widespread and weakened in the NTS group than the NNS group (see Fig. 2B).

3.3. Further analyses

Further analyses were performed to validate the findings obtained with the risk difference analyses performed. In particular, a false discovery rate (FDR) analysis (Genovese et al., 2002) was applied to the whole brain 90 × 90 binarized correlation matrix of functional connectivities.

For the NTS group, the majority of the links shown in Table 2 as significant in the risk difference analysis resulted in significant effects in the same direction with the FDR analysis with $q = 0.05$ (the one-tailed significance level given the prior hypothesis from the permutation test) for which the statistical threshold was $p = 0.0260$. For example, the following links were confirmed as significantly different between the NTS and the control groups using the FDR approach: INS.R–PUT.R; TPOmid.L–IFGtriang.R; IFGtriang.L–IFGtriang.R; PCG.L–PCUN.L; AMYG.L–AMYG.R; PreCG.L–PoCG.L; PreCG.L– PreCG.R; PAL.L–PAL.R; HIP.L–HIP.R; PHG.L–PHG.R. This additional analysis thus helps to validate some of the main conclusions of the paper, including the reduced functional connectivity for the PCG.L–PCUN.L link ($p = 0.003$ in the FDR analysis) in the NTS patients, and some of the reduced interhemispheric links including those between the two amygdalae, and the two temporal poles.

For the FDR analysis in the NNS group the statistical threshold was $p = 0.0081$ as the number of patients was smaller, but nevertheless the functional connectivities shown in Table 2 for the NNS group that were significantly different from controls included the following, in line with the risk-difference analyses: HIP.L-PHG.R; ROL.R-STG.R; SFGdor.L-MFG.L; MFG.R-IPL.R; and IFGoperc.L- IFGtriang.L.

We performed an additional analysis to validate the identification of the links in Table 2 that were common between the NTS and NNS patents as being reduced relative to controls. We did this by performing a statistical test that was whole brain corrected using FDR to identify which links in the combined NTS and NNS groups (83 patients) were different from the links in the controls. The FDR threshold for significance at $p < 0.05$ (two-tailed) across the whole brain was 0.0166, and all four common links were more significant than this threshold value of significance as follows. PCG–PCUN $p = 4.18 \times 10^{-4}$; AMYG.L-

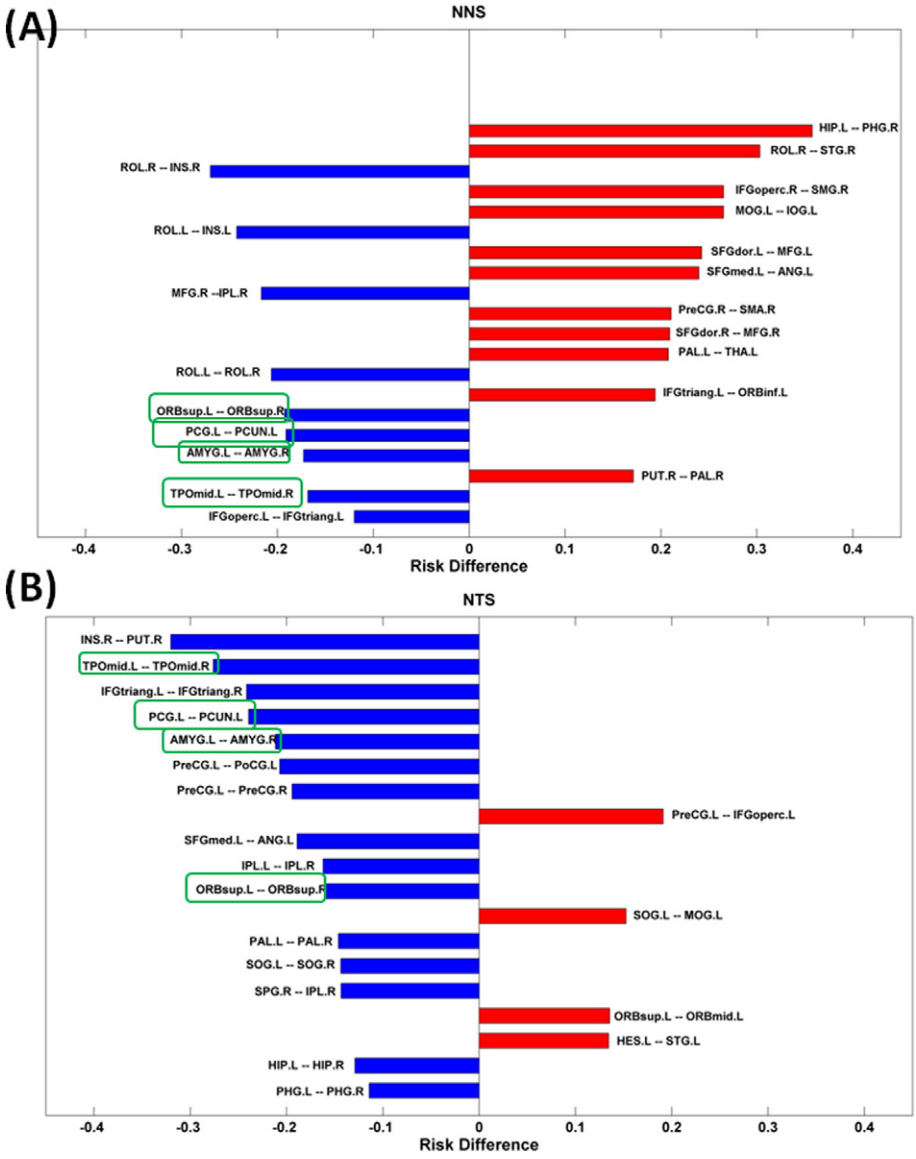

Fig. 1. A. Bar plot of the risk difference scores of the functional connectivity links in NNS patients relative to healthy controls. Negative scores reflect weaker links and are shown in blue, while positive scores reflect stronger links and are shown in red. B. The corresponding plots for the NTS patient group. Common altered links in NNS and NTS patient groups are marked with green rectangles.

AMYG.R $p = 2.5 \times 10^{-4}$; TPOmid.L-TPOmid.R p = 0.0012; ORBsup.L-ORBsup.R p = 0.02. Thus all four links shown as common in being reduced in NTS and NNS patients in Table 2 were found to be different using a whole brain FDR correction performed on the combined NNS and NTS groups vs controls. This is useful validation, for the links include links relevant to the association between avolition and the PCG–PCUN link described in the main text; and between the negative symptoms and the AMYG.L-AMYG.R, TPOmid.L-TPOmid.R, and the ORBsup.L-ORBsup.R links. To perform the correlations between these links and the symptoms, the following method was used. The correlation coefficient between the time series for two brain regions was replaced with a Fisher combined probability calculated across the Fourier coefficients of the correlations between the 2 time series from the two regions being analyzed (He et al., 2015). In more detail, we converted the 170-point time series with samples every 2 s from each subject into 17 segments each 10 time-points long, and calculated the Pearson correlation for each segment. The Fourier coefficients of this 17-segment time series of Pearson correlations was calculated. This algorithm provides a measure of the variation of the link correlations across the segments of the resting state time series. The Fourier coefficients that maximized the canonical correlation with the symptoms were used. In practice, the use of two Fourier coefficients did show significant correlations with the symptoms for the the PCG.L–PCUN.L link, as shown in Table 3.

4. Discussion

Features of the present study are that it used a whole-brain functional connectivity approach, and that it compared untreated patients with first-episode schizophrenia (NNS), with patients with first-episode schizophrenia treated with neuroleptic drugs (NTS), and with controls.

First we discuss the brain functional connectivity changes that were common to the two patient groups, involving the weakened PCG–PCUN coupling and some weakened interhemispheric couplings, as they are of great interest for understanding the neuropathophysiology of schizophrenia.

The PCG (posterior cingulate gyrus) and PCUN (precuneus), broadly known as the key nodes in the so-called "default mode network" (DMN) (Buckner et al., 2008; Fransson and Marrelec, 2008), are both located in the posterior medial parietal area. Importantly, within this area, the PCG and PCUN are reciprocally and strongly anatomically interconnected

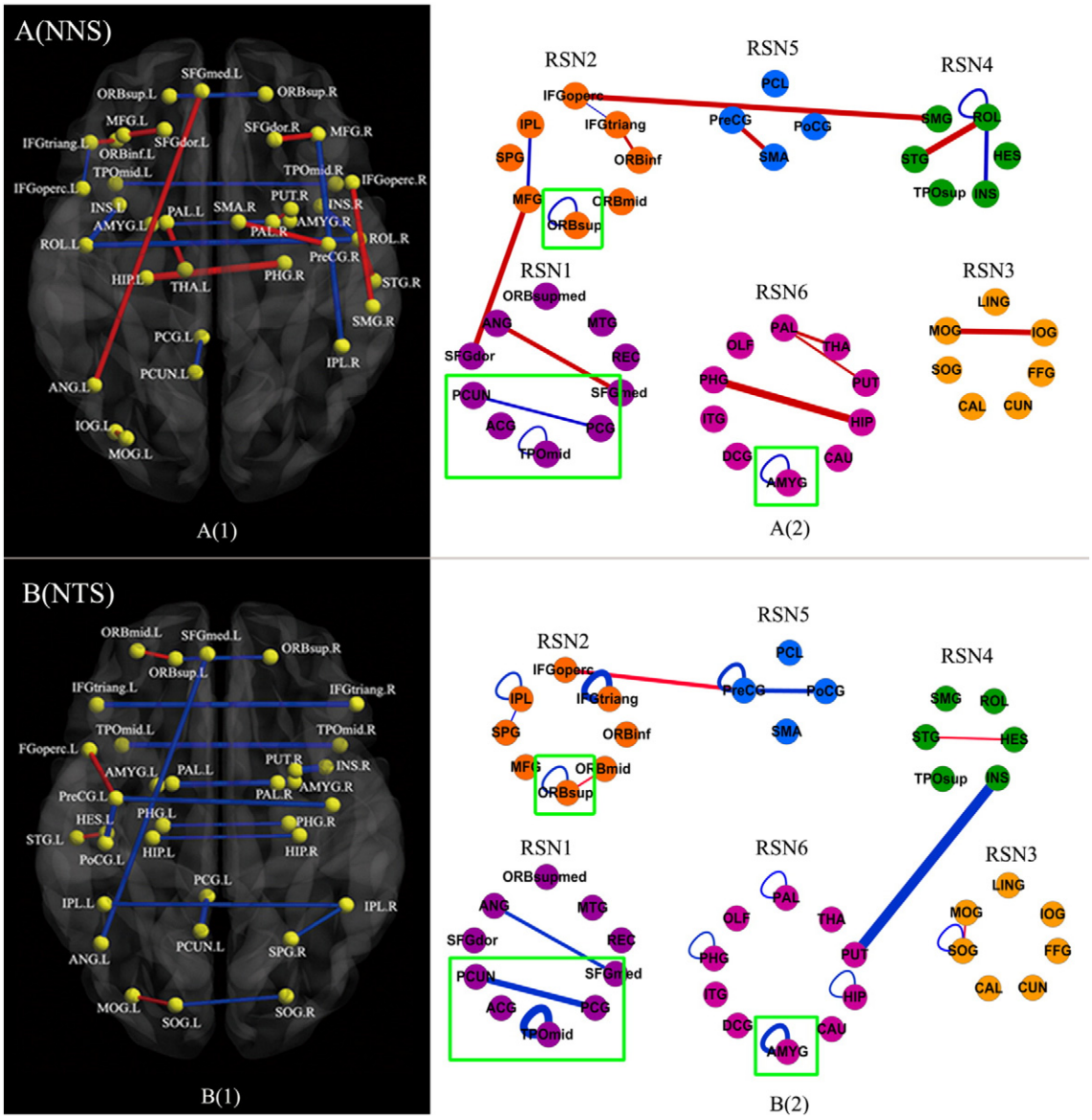

Fig. 2. Panels A and B show the altered links in neuroleptic-naïve patients (NNS) and neuroleptic-treated patients (NTS) relative to the healthy controls (HC) respectively: A(1) and B(1) show the locations of these altered links in a transverse plane of the brain; A(2) and B(2) show the locations of these altered links in six communities of resting state networks (RSN) in different colors. Twenty links in the NNS and nineteen links in the NTS patients were found to be altered. Red lines: links increased in patients; blue lines: links reduced in patients; looped links are to the contralateral side. Line thickness corresponds to the score s. The common altered links in NNS and NTS patients are marked with green rectangles.

(Cavanna and Trimble, 2006; Vogt, 2009), providing a robust anatomical basis for their functional connectivity analyzed here. Consistent with our finding, there have been many resting-state and task-related fMRI studies showing abnormal functions in the PCG and PCUN (Bluhm et al., 2007; Lynall et al., 2010; Whitfield-Gabrieli et al., 2009). Our subsequent source location analysis (Fig. 3) found that the exact voxels with altered connectivity in the PCG and PCUN in the NNS patients were quite similar to those in the NTS patients, confirming that the PCG–PCUN circuit changed commonly in the two patient groups. Therefore, it may be inferred that the reduced functional connectivity between PCG and PCUN, identified in both the treated and untreated patients in the present study, has extended previous evidence that the functional deficits in the PCG–PCUN circuit are not only critical in the pathophysiology of schizophrenia, but also are possibly resistant to current neuroleptic drugs. Consistent with this view, the altered functional connectivity in the default mode network in schizophrenia was not changed by 6 weeks of antipsychotic treatment (Lui et al., 2010).

Our subsequent correlation analysis showed a significant association between the weakened PCG–PCUN link and the reduced volition. Disturbances of volition as measured in this investigation using the PANSS scales include disturbances of the self-initiation, maintenance, and control of one's speech, thoughts, movements and behavior (Kay et al., 1987). That is, avolition in this sense describes a disturbance of the way in which the self is normally perceived as being involved as the agent that initiates speech, thoughts, movements and behavior. The precuneus and the posterior cingulate cortex have been described as being involved in consciousness of the self, and as having high activity when humans are engaged in self-related mental representations during rest (Cavanna and Trimble, 2006). This evidence is consistent with and supports our finding that functional impairments in the PCG/PCUN region are correlated in schizophrenia with dysfunctions in the sense of self and self-control (Northoff and Bermpohl, 2004), such as the volitional deficits. Consistent with this proposal, the volitional deficits shown by patients with damage to various parts of the cingulate gyrus have been related to impairment of the core self (Damasio, 2000). Indeed, recent fMRI studies have revealed abnormal brain activity and reduced functional connectivity in the PCG and PCUN in schizophrenic patients during self-referential processing and the resting state (Bluhm et al., 2007; Guo

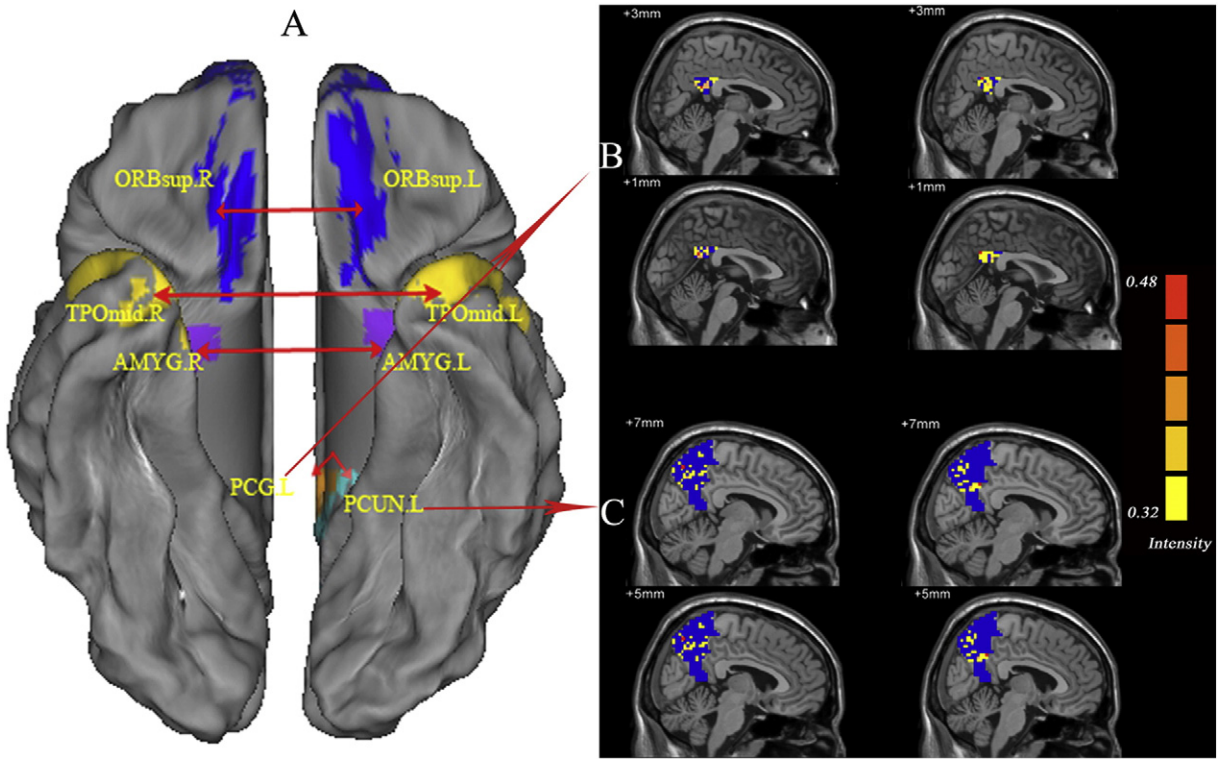

Fig. 3. Source location of the common altered links in neuroleptic-naïve and neuroleptic-treated schizophrenia patients. Panel A shows the locations of the common altered links in both patient groups on a brain template. Panels B and C show the source locations of the altered voxels in PCG and PCUN respectively, in which the right column illustrates the locations for NNS group, and the left column for the NTS group. Warm colors show is the altered voxels in the PCG and PCUN, and the blue colors show the unchanged voxels relative to healthy controls. The center voxels in the PCG and PCUN are denoted by red dots.

et al., 2014; Kuhn and Gallinat, 2013; Lynall et al., 2010; van Buuren et al., 2010), providing further evidence of the finding reported here of reduced volition associated with reduced functional connectivity between the PCG and PCUN in untreated patients with schizophrenia and also in patients treated with antipsychotics. An implication is that a treatment other than conventional antipsychotic drugs is needed to restore the sense of self and of control in schizophrenia. It was further found that the reduced functional connectivity in the PCG.L–PCUN.L link was associated with other general symptoms (including the general subtotal score of the PANSS) and with several of the negative symptoms of schizophrenia, but not with the positive symptom subtotal score of the PANSS (Table 3). The same observation applies that treatments other than conventional antipsychotic drugs may be needed to treat these other general and negative symptoms of schizophrenia (Rolls, 2012; Rolls et al., 2008).

Another important common feature of the functional alteration in the two patient groups is the reduced interhemispheric connectivity involving bilaterally the amygdalae, orbitofrontal cortex, and temporal pole. This is of interest, for it has been proposed that the lateralized abnormality in schizophrenia can be explained either by hemisphere dysfunction, or by impaired interhemispheric coupling, leading to a failure of the dominant hemisphere to over-rule non-dominant homologous areas (Annett, 1997; Bleich-Cohen et al., 2012). Functional connectivity analyses have demonstrated interhemispheric disconnection in the language cortex, particularly Broca's area in the prefrontal cortex (Bleich-Cohen et al., 2009a; Bleich-Cohen et al., 2009b). More evidence for this long-standing abnormal asymmetry hypothesis in schizophrenia comes from tract-tracing and automated imaging studies, which have consistently demonstrated the impaired integrity of white matter (WM) fibers interconnecting the two hemispheres, such as the corpus callosum (CC) and anterior commissure (AC) (Choi et al., 2011; Highley et al., 1999; Hulshoff Pol et al., 2004; Patel et al., 2011). In particular, the AC, which connects between the hemispheres the amygdalae, anterior temporal area, and the orbitofrontal cortex (Di Virgilio et al., 1999; Highley et al., 1999), has been reported to be impaired in both fiber density and WM integrity by post-mortem and diffusion tensor imaging (DTI) studies in schizophrenia (Choi et al., 2011; Highley et al., 1999). It is interesting to note that our finding of these weakened interhemispheric couplings has extended the evidence from anatomical disconnection to functional disconnection in the areas interconnected by the AC.

Table 3
Associations of clinical variables with the common altered links in both patient groups.

ROIs	Clinical variables	Correlation	p-Value
PCG. L – PCUN.L	PANSS general sum score	0.445	0.0015
	PANSS negative score	0.454	0.0011
	Disturbance of volition G13	0.448	0.002
	Uncooperativeness G8	0.623	1.3×10^{-6}
	Unusual thought content G9	0.455	0.001
	Lack of judgement and insight G12	0.488	0.0006
	Hostility P7	0.5853	9.7×10^{-6}

Note: see names and abbreviations of the region of interest in Table S1.
All the results shown were significant with $p < 0.05$ after FDR corrected for multiple comparisons.

Furthermore, the impaired interhemispheric functional connectivity involving the amygdalae, anterior temporal area, and the orbitofrontal cortex may be related to the negative symptoms in schizophrenia, as these regions are involved in emotion and motivation (Rolls, 2013, 2014a). Importantly, previous studies have suggested little effectiveness of pharmacological treatments on the negative symptoms (Kane, 1999; Rosenheck et al., 1999; Smith et al., 1996). Thus new agents targeting these interhemispheric uncouplings involved in emotional and motivational processing may help towards better functional recovery for patients with schizophrenia.

Taken together, these common brain alterations in the NNS and NTS patients, in combination with their associations with volitional deficits and negative symptoms, suggest current pharmacological treatment may have less efficiency on the volitional deficits and negative symptoms, and their related neurophysiological deficits in the schizophrenia patients. These points thus lead us to suggest that our findings may provide

a clue for new pharmacological agents to target the negative symptoms and volitional deficits and their related neurophysiological substrates in schizophrenia.

The present study, by applying a whole-brain functional connectivity approach, also showed different patterns of functional deficits between the untreated and treated patients, which may provide further insight into the medication effects on the functional connectivity in the brain of patients with schizophrenia. We found that in the NNS patients, functional connectivity was increased in hippocampal (PHG-HIP) and auditory cortical (STG) links, and in fronto-parietal circuits (the SFGdor-MFG-IPL circuit and ORBinf-IFGoper-IFGtriang-SMG circuit) and subcortical circuits (the striato-thalamic circuit and hippocampo-parahippocampal circuit), and became closer to normal in the NTS patients (Fig. 1). The hippocampal/temporal lobe connectivity may be related to the positive symptoms of schizophrenia, the fronto-parietal circuits may be related with the deficits of decision-making, working memory and attention in schizophrenia (Tan et al., 2006), and the strengthened subcortical striato-thalamic circuit is believed to be closely related to the pathology of schizophrenia (Menon et al., 2001) and to the effects of antipsychotic drugs (Heinz and Schlagenhauf, 2010). These findings are consistent with a follow-up resting-state fMRI study, in which it was found that the abnormal frontoparietal and striatal functions were normalized by antipsychotic medication for 6 weeks (Lui et al., 2010). This restoration by the dopamine D2 receptor blockade produced by anti-psychotic treatment may facilitate the effects of GABA, and thus reduce the increased functional connectivity found in these circuits in the untreated patients with schizophrenia (Rolls, 2012; Rolls et al., 2008). However, although altered connectivity of the superior temporal gyrus (STG) has been consistently demonstrated as correlated with hallucinations in schizophrenia (Hoffman and Hampson, 2011; Shinn et al., 2013), in the present study, hallucinations were not different between the NNS (Mean ± SD: 3.84 ± 1.31) and NTS (Mean ± SD: 3.56 ± 1.61) patients.

However, although altered connectivity of the superior temporal gyrus (STG) has been consistently demonstrated as correlated with hallucinations in schizophrenia (Plaze et al., 2006; Pu et al., 2012), in the present study, hallucinations were not different between the NNS (Mean ± SD: 3.84 ± 1.31) and NTS (Mean ± SD: 3.56 ± 1.61) patients.

The present study applying a whole-brain functional connectivity analysis enables us to reveal a broadly distributed dysconnectivity pattern across large-scale brain networks in schizophrenia, lending further support to the notion of a diffuse dysregulation of neural dynamics in this disorder (Fornito et al., 2012). Our observation, that most of the increased functional connectivity in the neuroleptic-naïve (NNS) patients appears to be normalized in the neuroleptic-treated (NTS) patients, has highlighted the important potential contribution of medication effects to the conflicting findings of both increased and decreased functional connectivity in previous studies (Pettersson-Yeo et al., 2011). Moreover, at the brain-network level the impaired circuits in the posterior Default Mode Network and emotion-processing system that were not influenced by treatment with neuroleptics may provide new drug targets for the negative symptoms, which are not treated well with current antipsychotics. Additional implications of the present study for future studies include the following. First, our neuroleptic-treated patients showed only a trend towards reduction of the positive symptoms compared to the untreated patients, so in future studies it could be of interest to study patients at different stages of their treatment with neuroleptics. (In the present study, 21 NTS patients had an illness duration of less than 6 months, and 28 had an illness duration of less than 12 months. The duration of treatment of the NTS group was in the range 2–144 weeks, with a median of 32 weeks.) Second, the clinical symptoms of the schizophrenic patients in the present study were measured with the PANSS (Kay et al., 1987). The three-factor structure (Positive, Negative and General Psychopathology Symptoms) of the PANSS, however, may need some revision. For example, for the "disturbance of volition" included as G13, a body of evidence has suggested that this item should be considered with the Negative symptoms (Emsley et al., 2003; Kay and Sevy, 1990; Lancon et al., 1999; Lykouras et al., 2000), and may be similar to the "avolition" in another psychopathology assessment scale SANS (Andreasen, 1982). Moreover, the description of G13 appears to emphasize indecisiveness which may not relate only to volitional deficits but also to cognitive impairments such as disrupted attentional control. Thus, further research on the link between volition and PCC-PCUN functional connectivity might incorporate more detailed measures of volition. Third, in the present study we were not able to compare the effects on functional connectivity of typical and atypical neuroleptics, as only 9 patients received first generation (typical) antipsychotic medication, and this comparison might be of interest for future studies.

In conclusion, the present study, by applying a whole-brain functional connectivity approach, revealed decreased functional connectivity in the precuneus–parahippocampal connectivity that was associated with the decrease in the sense of the self being in control (decreased volition); and a decrease in interhemispheric connectivity between temporal lobe structures involved in emotion that was related to the negative symptoms of schizophrenia. This reduced connectivity, and the related volition and negative symptoms, were not treated by the antipsychotic drugs used in the NTS group. An implication is that treatments that increase this connectivity, such as treatments that restore the hypoglutamatergia of schizophrenia (Coyle, 2012; Coyle et al., 2012), might treat these symptoms of schizophrenia (Rolls, 2012; Rolls et al., 2008). The increased functional connectivity found in hippocampal and auditory circuits may be related to the positive symptoms of schizophrenia, and were reduced by the antipsychotic treatment, which may have produced this change by the D2 receptor blockade which can facilitate the actions of the inhibitory transmitter GABA (Rolls, 2012; Rolls et al., 2008). In addition, the results described here inform our basic understanding of brain structure–function relations by providing new evidence on the relation between the precuneus and posterior cingulate gyrus and volition, understood as the willful initiation, sustenance, and control of thoughts, behavior, movements, and speech; and on how reduced interhemispheric functional connectivity between brain structures such as the amygdala, orbitofrontal cortex, and temporal pole could be related to reductions in emotion and motivation that relate to the negative symptoms of schizophrenia, emotional withdrawal, and depression.

Acknowledgments

This research was supported by grants from the National 973 Program of China (2011CB707800 to Dr. ZN Liu), National Natural Science Foundation of China (81271485 to Dr. ZN Liu, 81071092 to Dr. ZN Liu, 30971053 to Dr. ZM Xue, and 11271121 to Dr. SX Guo, 81000587 to Dr. HH Liu), Specialized Research Fund for the Doctoral Program of Higher Education (20110162110017 to Dr. ZN Liu), Program for New Century Excellent Talents in University (NCET-13-0786 to Dr. SX Guo), and National Centre for Mathematics and Interdisciplinary Science (NCMIS) in Chinese Academy of Sciences (to Prof JF Feng). Support was also received from the Oxford Centre for Computational Neuroscience (ETR). The authors thank Dr. Wenlian Lu of the University of Warwick for making code available for the analysis of functional connectivity using Fourier analyses of the time series (Lu et al., 2014). National Natural Science Foundation (81401125 to Dr. W.D.Pu); Natural Science Foundation of Hunan Province (2015JJ1010 to Dr S.Guo).

Appendix

Inclusion and Exclusion Criterion for the Participants

Twenty-two neuroleptic-naïve schizophrenic patients (NNS) and sixty-one neuroleptic-treated schizophrenia patients (NTS) were recruited from the Department of Psychiatry of the Second Xiangya Hospital of Central South University, Changsha, China. All participants were diagnosed with schizophrenia using the Structural Clinical

Interview for DSM-IV, Patient version (SCID-I/P) and met the following inclusion criteria: 1) 18-45 years of age; 2) Han Chinese ethnicity; and 3) right handedness. Participants were excluded if they had: 1) a history of substance-related disorders; 2) a history of neurological illness or other serious physical illness; 3) a contraindication for MRI; or 4) a history of electroconvulsive therapy. The patients in the two groups were similar, except that the research described here was performed in the NNS group before treatment started.

Sixty healthy controls (HC), including 35 males and 25 females, were recruited from Changsha, China. The inclusion and exclusion criteria for HC were the same as those for the schizophrenic patients other than the DSM-IV diagnostic criteria. Individuals with a family history of psychiatric illness among their first-degree relatives were excluded from the HC group.

The PANSS (Kay et al., 1987) sum scores for the NNS vs NTS groups showed no significant differences, and for the PANSS positive sum score were 22.5±6.2 vs 19.0±6.2 (mean±sd); for the PANSS negative sum score were 22.7±10.3 vs 21.2±6.7; for the PANSS general psychiatric sum score were 42.4±14.9 vs 38.2±10.5; and for the PANSS sum score were 92.3±31.8 vs 85.1±20.3). It is noted that the positive symptom sum score showed a trend-level difference (t=-1.89, p=0.06) between the two patient groups.

Medication Dosage and Duration of Neuroleptic-treated Patients

All the NTS patients were taking antipsychotics at the time of scanning: nine patients (15%) were receiving first generation antipsychotics (FGAs) (sulpiride, 7 cases; and haloperidol, 2 cases); forty two patients (69%) were receiving second generation antipsychotics (SGAs) (clozapine, 7 cases; risperidone, 19 cases; quetiapine, 10 cases; olanzapine, 6 cases), and ten patients (16%) were receiving combination therapy (combining an FGA and an SGA, 4 cases; and combining an SGA and an SGA, 6 cases). All medication doses were converted to chlorpromazine equivalence (50~1500 mg/day). The mean dosages of treatments with FGAs, SGAs and combination therapy were 822.9 mg/day, 384.6 mg/day and 572.3 mg/day respectively. Moreover, the mean medication duration of the NTS patients was 36.8 weeks (2 ~144 weeks).

Image acquisition

All subjects underwent structural and functional MRI scanning using the same 1.5-T GE Signa Twinspeed MR scanner (General Electric Medical System, Milwaukee, USA). A standard head coil was used for radio frequency transmission and reception of the nuclear magnetic resonance signal. Foam pads and ear plugs were used to minimize head motion and scanner noise. All the subjects were instructed to keep their eyes closed, not to think about anything in particular and to move as little as possible. 3-D structural MRI images (T1-weighted) were acquired from the sagittal plane using spoiled gradient echo (SPGR) pulse sequence, scanning parameter: TR = 12 ms, TE = 4.2 ms, flip angle = 15°, 172 slices, matrix size = 256 × 256, and the field of view (FOV) = 24×24 cm. Slices were contiguous with slice thickness of 1.8mm. Functional images were acquired by using a gradient-echo echo-planar imaging sequence sensitive to BOLD signal (TR/TE = 2000/40 ms, flip angle = 90°, FOV = 24×24 cm). Whole-brain volumes were acquired with 20 contiguous 5mm thick transverse slices with a 1mm gap and 3.75×3.75 mm in-plane resolution. For each subject, fMRI scanning lasted for 6 minutes and 180 volumes were obtained.

Functional Imaging data preprocessing

Before preprocessing, the first 10 volumes were discarded to allow for scanner stabilization and the subjects' adaptation to the environment. fMRI data preprocessing was then conducted by SPM5 (University College London, UK; http://www.fil.ion.ucl.ac.uk/spm) and DPARSF (Data Processing Assistant for resting-state fMRI). The remaining functional scans were quickly corrected for within-scan acquisition time differences between slices, and then realigned to the middle volume to correct for interscan head motion. Subsequently, the functional scans were spatially normalized to a standard template (Montreal Neurological Institute) and re-sampled to 3 × 3 × 3 mm. After normalization, the Blood Oxygenation Level Dependent (BOLD) signal of each voxel was first detrended to remove the linear trend and then passed through a band-pass filter (0.01-0.08 Hz) to reduce low-frequency drift and high-frequency physiological noise. In addition to band-pass filtering and correcting for movements, additional preprocessing steps, such as global signal regression (Fox et al., 2009), were performed. Global signal regression may lead to artefactual negative correlations, but this technique was used because it is suggested to improve the specificity of positive correlations and can remove specific confounds from the data to facilitate the evaluation of neurophysiological relationships, helping to make the data more readily or reliably interpretable (Fox et al., 2009). We note that there is recent evidence that the global brain signal is altered in schizophrenia (Yang et al., 2014). Thus, nuisance covariates, including head motion parameters, global mean signals, white matter signals and cerebrospinal fluid signals were regressed out from the Blood Oxygenation Level Dependent (BOLD) signals.

Voxel Location

In order to detect the location of the voxels in the PCG and PCUN regions which show significant differences between patients and healthy controls (Fig. 3), Pearson correlation coefficients between all pairs of voxels of the PCG-PCUN link were first calculated, then a Fisher's r-to-z transformation was utilized to convert each correlation coefficient r_{ij} into Z_{ij} to improve the normality. After Fisher's r-to-z transformation, two-sample t-tests were performed for all inter-voxel correlation coefficients. A significance level of $p < 0.05$ was used to specify the source voxels within this regional link. To identify dysfunctional voxels, for each selected voxel i in region A, a dysfunction intensity for this voxel was defined as follows:

$$Intensity(i) = \frac{N_i^B}{N^B}$$

where N_i^B is the number of voxels in region B that show significantly different ($p<0.05$) functional connectivity with the selected voxel i in region A compared with normal controls. N^B is the total number of voxels in region B. This is reasonable since the value of intensity represents the significance of the changed correlation for each voxel. An intensity level (intensity>0.32) was further used to threshold voxels into two groups of voxels (unchanged and changed).

Table S1
The Names and Abbreviations of the Regions of Interest (ROIs).

Regions	Abbr.	Regions	Abbr.
Amygdala	AMYG	Orbitofrontal cortex (middle)	ORBmid
Angular gyrus	ANG	Orbitofrontal cortex (superior)	ORBsup
Anterior cingulate gyrus	ACG	Pallidum	PAL
Calcarine cortex	CAL	Paracentral lobule	PCL
Caudate	CAU	Parahippocampal gyrus	PHG
Cuneus	CUN	Postcentral gyrus	PoCG
Fusiform gyrus	FFG	Posterior cingulate gyrus	PCG
Heschlgyrus	HES	Precentral gyrus	PreCG
Hippocampus	HIP	Precuneus	PCUN
Inferior occipital gyrus	IOG	Putamen	PUT
Inferior frontal gyrus (opercula)	IFGoperc	Rectus gyrus	REC

(continued on next page)

Table S1 *(continued)*

Regions Abbr.		Regions	Abbr.
Inferior frontal gyrus(triangular)	IFGtriang	Rolandic operculum	ROL
Inferior parietal lobule	IPL	Superior occipital gyrus	SOG
Inferior temporal gyrus	ITG	Superior frontal gyrus (dorsal)	SFGdor
Insula	INS	Superior frontal gyrus (medial)	SFGmed
Lingual gyrus	LING	Superior parietal gyrus	SPG
Middle cingulate gyrus	MCG	Superior temporal gyrus	STG
Middle occipital gyrus	MOG	Supplementary motor area	SMA
Middle frontal gyrus	MFG	Supramarginal gyrus	SMG
Middle temporal gyrus	MTG	Temporal pole (middle)	TPOmid
Olfactory	OLF	Temporal pole (superior)	TPOsup
Orbitofrontal cortex (inferior)	ORBinf	Thalamus	THA
Orbitofrontal cortex (medial)	ORBmed		

Table S2
Significant Differences of PANSS Sub-scales between Neuroleptic-naïve Patients and Neuroleptic-treated Patients.

Variables	NNS (N=22)		NTS (N=61)	
	Mean	SD	Mean	SD
Delusion score	4.8	2.0	3.7	1.4
Hostility score	2.6	1.1	1.8	1.0
Stereotyped thinking score	2.3	1.3	1.5	0.7
Anxiety score	3.4	1.3	2.6	1.1
Tension score	3.0	1.4	2.3	1.0
mannerisms and posturing score	2.7	1.5	2.0	0.9
Uncooperativeness score	2.4	1.2	1.6	0.9
Poor impulse control score	3.4	1.6	2.4	1.2
Preoccupation score	3.1	1.4	2.3	1.2

Note: NNS, neuroleptic-naïve schizophrenia; NTS neuroleptic-treated schizophrenia; PANSS, positive and negative syndrome scale; SD, standard deviation.

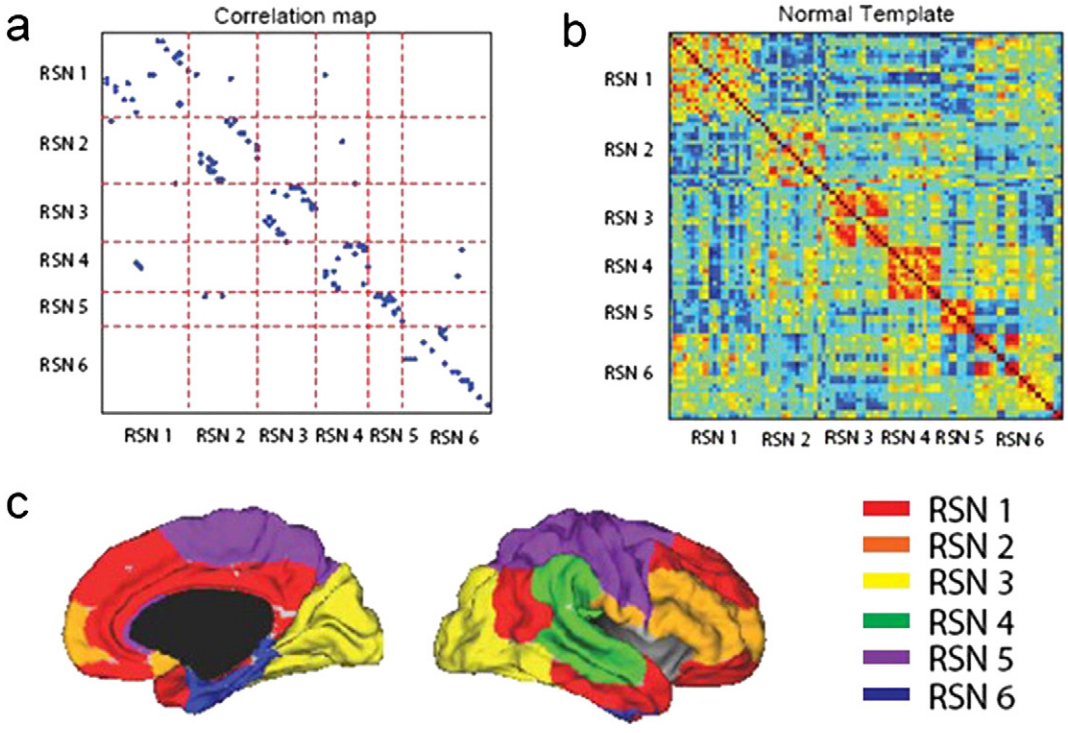

Fig. S1. a. Community structure derived from normal healthy subjects (Tao et al., 2013). b. The correlation coefficient matrix of the BOLD signals from 90 ROIs of one randomly selected subject. c. (Left) Medial view of the surface of the brain. (Right) The lateral view of the surface of the brain. Different colors represent different communities.

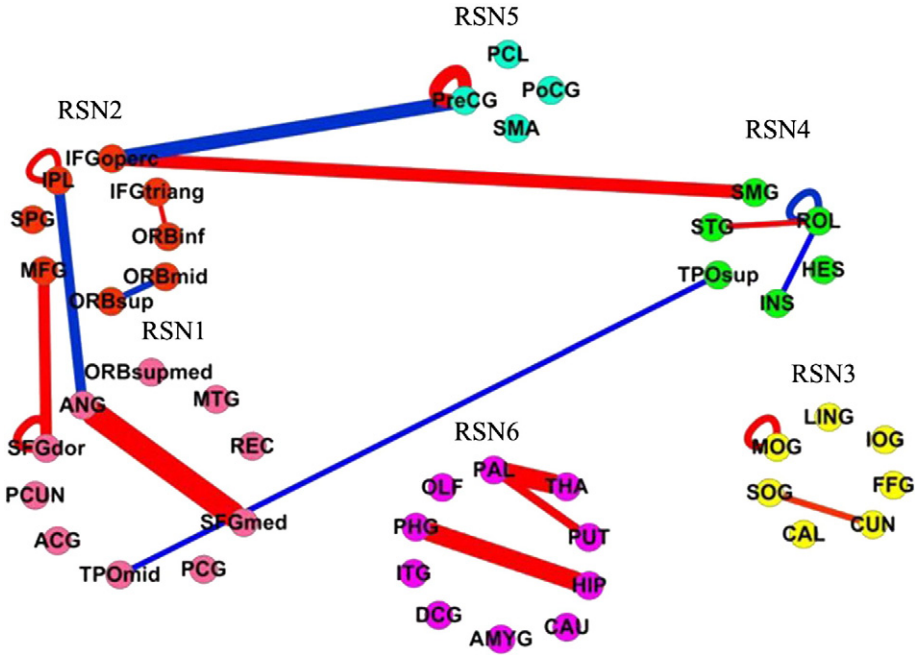

Fig. S2. Differences in functional connectivity maps between neuroleptic-naïve and neuroleptic-treated schizophrenia patients. The red lines indicate links strengthened in NNS patients and blue lines indicate links weakened in NNS patients, compared to NTS patients. The line thickness indicates the difference magnitude of the connection strength between the two patient groups. The common altered links in the two patient groups shown include the precuneus-posterior cingulate cortex link, and three interhemispheric links (bilateral orbitofrontal cortex, amygdalae and temporal pole). These links showed no significant differences ($p>0.05$) between the two patient groups.

References

Achard, S., Bullmore, E., 2007. Efficiency and cost of economical brain functional networks. PLoS Computational Biology 3 (2), e17. http://dx.doi.org/10.1371/journal.pcbi.003001717274684.

Andreasen, N.C., 1982. Negative symptoms in schizophrenia. Definition and reliability. Archives of General Psychiatry 39 (7), 784–788. http://dx.doi.org/10.1001/archpsyc.1982.04290070020005716547 7.

Annett, M., 1997. Schizophrenia and autism considered as the products of an agnosic right shift gene. Cognitive Neuropsychiatry 2 (3), 195–240. http://dx.doi.org/10.1080/135468097396333165714 95.

Bleich-Cohen, M., Hendler, T., Kotler, M., Strous, R.D., 2009a. Reduced language lateralization in first-episode schizophrenia: an fMRI index of functional asymmetry. Psychiatry Research 171 (2), 82–93. http://dx.doi.org/10.1016/j.pscychresns.2008.03.00219185468.

Bleich-Cohen, M., Sharon, H., Weizman, R., Poyurovsky, M., Faragian, S., Hendler, T., 2012. Diminished language lateralization in schizophrenia corresponds to impaired interhemispheric functional connectivity. Schizophrenia Research 134 (2-3), 131–136. http://dx.doi.org/10.1016/j.schres.2011.10.01122115994.

Bleich-Cohen, M., Strous, R.D., Even, R., Rotshtein, P., Yovel, G., Iancu, I., Olmer, A., Hendler, T., 2009b. Diminished neural sensitivity to irregular facial expression in first-episode schizophrenia. Human Brain Mapping 30 (8), 2606–2616. http://dx.doi.org/10.1002/hbm.2069619172653.

Bluhm, R.L., Miller, J., Lanius, R.A., Osuch, E.A., Boksman, K., Neufeld, R.W., Théberge, J., Schaefer, B., Williamson, P., 2007. Spontaneous low-frequency fluctuations in the BOLD signal in schizophrenic patients: Anomalies in the default network. Schizophrenia Bulletin 33 (4), 1004–1012. http://dx.doi.org/10.1093/schbul/sbm05217556752.

Bowie, C.R., Reichenberg, A., Patterson, T.L., Heaton, R.K., Harvey, P.D., 2006. Determinants of real-world functional performance in schizophrenia subjects: correlations with cognition, functional capacity, and symptoms. American Journal of Psychiatry 163 (3), 418–425. http://dx.doi.org/10.1176/appi.ajp.163.3.41816513862.

Buckner, R.L., Andrews-Hanna, J.R., Schacter, D.L., 2008. The brain's default network: anatomy, function, and relevance to disease. Annals of the New York Academy of Sciences 1124, 1–38. http://dx.doi.org/10.1196/annals.1440.01118400922.

Cavanna, A.E., Trimble, M.R., 2006. The precuneus: a review of its functional anatomy and behavioural correlates. Brain: A Journal of Neurology 129 (3), 564–583. http://dx.doi.org/10.1093/brain/awl00416399806.

Cheng, W., Ji, X., Zhang, J., Feng, J., 2012. Individual classification of ADHD patients by integrating multiscale neuroimaging markers and advanced pattern recognition techniques. Frontiers in Systems Neuroscience 6, 58. http://dx.doi.org/10.3389/fnsys.2012.00058228883 14.

Choi, H., Kubicki, M., Whitford, T.J., Alvarado, J.L., Terry, D.P., Niznikiewicz, M., McCarley, R.W., Kwon, J.S., Shenton, M.E., 2011. Diffusion tensor imaging of anterior commissural fibers in patients with schizophrenia. Schizophrenia Research 130 (1-3), 78–85. http://dx.doi.org/10.1016/j.schres.2011.04.01621561738.

Coyle, J.T., 2012. NMDA receptor and schizophrenia: a brief history. Schizophrenia Bulletin 38 (5), 920–926. http://dx.doi.org/10.1093/schbul/sbs07622987850.

Coyle, J.T., Basu, A., Benneyworth, M., Balu, D., Konopaske, G., 2012. Glutamatergic synaptic dysregulation in schizophrenia: therapeutic implications. Handbook of Experimental Pharmacology 267–295 http://dx.doi.org/10.1007/978-3-642-25758-2_1023027419.

Damasio, A., 2000. The Feeling of What Happens: Body and Emotion in the Making of Consciousness. Harvest Books.

Di Virgilio, G., Clarke, S., Pizzolato, G., Schaffner, T., 1999. Cortical regions contributing to the anterior commissure in man. Experimental Brain Research 124 (1), 1–7. http://dx.doi.org/10.1007/s002210050593992878 3.

Emsley, R., Rabinowitz, J., Torreman, M., 2003. The factor structure for the Positive and Negative Syndrome Scale (PANSS) in recent-onset psychosis. Schizophrenia Research 61 (1), 47–5712648735.

First, M.B., Spitzer, R.L., Gibbon, M., 1996. Structured Clinical Interview for DSM-IV Axis I Disorder-Patient Edition (SCID-I/P). Biometrics Research Department, New York State Psychiatric Institute, New York.

Fornito, A., Zalesky, A., Pantelis, C., Bullmore, E.T., 2012. Schizophrenia, neuroimaging and connectomics. Neuroimage 62 (4), 2296–2314. http://dx.doi.org/10.1016/j.neuroimage.2011.12.09022387165.

Fox, M.D., Zhang, D., Snyder, A.Z., Raichle, M.E., 2009. The global signal and observed anticorrelated resting state brain networks. Journal of Neurophysiology 101 (6), 3270–3283. http://dx.doi.org/10.1152/jn.90777.200819339462.

Fransson, P., Marrelec, G., 2008. The precuneus/posterior cingulate cortex plays a pivotal role in the default mode network: evidence from a partial correlation network analysis. Neuroimage 42 (3), 1178–1184. http://dx.doi.org/10.1016/j.neuroimage.2008.05.05918598773.

Frith, C., 1995. Functional imaging and cognitive abnormalities. Lancet 346 (8975), 615–620. http://dx.doi.org/10.1016/S0140-6736(95)91441-27651009.

Genovese, C.R., Lazar, N.A., Nichols, T., 2002. Thresholding of statistical maps in functional neuroimaging using the false discovery rate. Neuroimage 15 (4), 870–878. http://dx.doi.org/10.1006/nimg.2001.103711906227.

Guo, S., Kendrick, K.M., Yu, R., Wang, H.L., Feng, J., 2014. Key functional circuitry altered in schizophrenia involves parietal regions associated with sense of self. Human Brain Mapping 35 (1), 123–139. http://dx.doi.org/10.1002/hbm.22162230081 70.

Hagmann, P., Cammoun, L., Gigandet, X., Meuli, R., Honey, C.J., Wedeen, V.J., Sporns, O., 2008. Mapping the structural core of human cerebral cortex. PLoS Biology 6 (7), e159. http://dx.doi.org/10.1371/journal.pbio.006015918597554.

Heinz, A., Schlagenhauf, F., 2010. Dopaminergic dysfunction in schizophrenia: salience attribution revisited. Schizophrenia Bulletin 36 (3), 472–485. http://dx.doi.org/10.1093/schbul/sbq03120453041.

He, X., Feng, J., Lu, W., 2015. A temporal-frequency feature selection method for small sample size. International Conference on Bio-inspired Systems and Signal Processing. Lisbon, Portugal.

Highley, J.R., Esiri, M.M., McDonald, B., Roberts, H.C., Walker, M.A., Crow, T.J., 1999. The size and fiber composition of the anterior commissure with respect to gender and schizophrenia. Biological Psychiatry 45 (9), 1120–1127. http://dx.doi.org/10.1016/S0006-3223(98)00323-010331103.

Hoffman, R.E., Hampson, M., 2011. Functional connectivity studies of patients with auditory verbal hallucinations. Frontiers in Human Neuroscience 6, 6. http://dx.doi.org/10.3389/fnhum.2012.0000622375109.

Honey, G.D., Suckling, J., Zelaya, F., Long, C., Routledge, C., Jackson, S., Ng, V., Fletcher, P.C., Williams, S.C., Brown, J., 2003. Dopaminergic drug effects on physiological connectivity in a human cortico-striato-thalamic system. Brain: A Journal of Neurology 126 (8), 1767–1781. http://dx.doi.org/10.1093/brain/awg18412805106.

Hulshoff Pol, H.E., Schnack, H.G., Mandl, R.C., Cahn, W., Collins, D.L., Evans, A.C., Kahn, R.S., 2004. Focal white matter density changes in schizophrenia: Reduced interhemispheric connectivity. Neuroimage 21 (1), 27–35. http://dx.doi.org/10.1016/j.neuroimage.2003.09.02614741639.

Insel, T.R., 2010. Rethinking schizophrenia. Nature 468 (7321), 187–193. http://dx.doi.org/10.1038/nature0955221068826.

Ji, X., Cheng, W., Ge, T., Zhang, J., Rolls, E.T., Sun, L., Wang, Y., Feng, J., ADHD: Increased coupling in the saliency network. Human Brain Mapping (2014) in revision

Kane, J.M., 1999. Pharmacologic treatment of schizophrenia. Biological Psychiatry 46 (10), 1396–1408. http://dx.doi.org/10.1016/S0006-3223(99)00059-110578454.

Kay, S.R., Fiszbein, A., Opler, L.A., 1987. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophrenia Bulletin 13 (2), 261–276. http://dx.doi.org/10.1093/schbul/13.2.2613616518.

Kay, S.R., Sevy, S., 1990. Pyramidical model of schizophrenia. Schizophrenia Bulletin 16 (3), 537–545. http://dx.doi.org/10.1093/schbul/16.3.5372287938.

Khamsi, R., 2012. Diagnosis by default. Nature Medicine 18 (3), 338–340. http://dx.doi.org/10.1038/nm0312-33822395690.

Kühn, S., Gallinat, J., 2013. Resting-state brain activity in schizophrenia and Major depression: a quantitative Meta-analysis. Schizophrenia Bulletin 39 (2), 358–365. http://dx.doi.org/10.1093/schbul/sbr15122080493.

Lançon, C., Reine, G., Llorca, P.M., Auquier, P., 1999. Validity and reliability of the French-language version of the Positive and Negative Syndrome Scale (PANSS). Acta Psychiatrica Scandinavica 100 (3), 237–243. http://dx.doi.org/10.1111/j.1600-0447.1999.tb10851.x10493091.

Laupacis, A., Sackett, D.L., Roberts, R.S., 1988. An assessment of clinically useful measures of the consequences of treatment. New England Journal of Medicine 318 (26), 1728–1733. http://dx.doi.org/10.1056/NEJM198806303182605337454545.

Lu, W., He, X., Feng, J., A framework for statistical testing of functional data (2014)

Lui, S., Li, T., Deng, W., Jiang, L., Wu, Q., Tang, H., Yue, Q., Huang, X., Chan, R.C., Collier, D.A., Meda, S.A., Pearlson, G., Mechelli, A., Sweeney, J.A., Gong, Q., 2010. Short-term effects of antipsychotic treatment on cerebral function in drug-naive first-episode schizophrenia revealed by "resting state" functional magnetic resonance imaging. Archives of General Psychiatry 67 (8), 783–792. http://dx.doi.org/10.1001/archgenpsychiatry.2010.8420679586.

Lykouras, L., Oulis, P., Psarros, K., Daskalopoulou, E., Botsis, A., Christodoulou, G.N., Stefanis, C., 2000. Five-factor model of schizophrenic psychopathology: how valid is it? European Archives of Psychiatry and Clinical Neuroscience 250 (2), 93–100. http://dx.doi.org/10.1007/s0040600700411085392 5.

Lynall, M.E., Bassett, D.S., Kerwin, R., McKenna, P.J., Kitzbichler, M., Muller, U., Bullmore, E., 2010. Functional connectivity and brain networks in schizophrenia. Journal of Neuroscience: the Official Journal of the Society for Neuroscience 30 (28), 9477–9487. http://dx.doi.org/10.1523/JNEUROSCI.0333-10.201020631176.

MacDonald, A.W., Schulz, S.C., 2009. What we know: findings that every theory of schizophrenia should explain. Schizophrenia Bulletin 35 (3), 493–508. http://dx.doi.org/10.1093/schbul/sbp01719329559.

Menon, V., Anagnoson, R.T., Glover, G.H., Pfefferbaum, A., 2001. Functional magnetic resonance imaging evidence for disrupted basal ganglia function in schizophrenia. American Journal of Psychiatry 158 (4), 646–649. http://dx.doi.org/10.1176/appi.ajp.158.4.64611282705.

Meyer-Lindenberg, A.S., Olsen, R.K., Kohn, P.D., Brown, T., Egan, M.F., Weinberger, D.R., Berman, K.F., 2005. Regionally specific disturbance of dorsolateral prefrontal–hippocampal functional connectivity in schizophrenia. Archives of General Psychiatry 62 (4), 379–386. http://dx.doi.org/10.1001/archpsyc.62.4.37915809405.

Milev, P., Ho, B.C., Arndt, S., Andreasen, N.C., 2005. Predictive values of neurocognition and negative symptoms on functional outcome in schizophrenia: a longitudinal first-episode study with 7-year follow-up. American Journal of Psychiatry 162 (3), 495–506. http://dx.doi.org/10.1176/appi.ajp.162.3.49515741466.

Mueser, K.T., McGurk, S.R., 2004. Schizophrenia. Lancet 363 (9426), 2063–2072. http://dx.doi.org/10.1016/S0140-6736(04)16458-115207959.

Northoff, G., Bermpohl, F., 2004. Cortical midline structures and the self. Trends in Cognitive Sciences 8 (3), 102–107. http://dx.doi.org/10.1016/j.tics.2004.01.00415301749.

Patel, S., Mahon, K., Wellington, R., Zhang, J., Chaplin, W., Szeszko, P.R., 2011. A meta-analysis of diffusion tensor imaging studies of the corpus callosum in schizophrenia. Schizophrenia Research 129 (2-3), 149–155. http://dx.doi.org/10.1016/j.schres.2011.03.01421530178.

Pettersson-Yeo, W., Allen, P., Benetti, S., McGuire, P., Mechelli, A., 2011. Dysconnectivity in schizophrenia: where are we now? Neuroscience and Biobehavioral Reviews 35 (5), 1110–1124. http://dx.doi.org/10.1016/j.neubiorev.2010.11.00421115039.

Plaze, M., Bartrés-Faz, D., Martinot, J.L., Januel, D., Bellivier, F., De Beaurepaire, R., Chanraud, S., Andoh, J., Lefaucheur, J.P., Artiges, E., Pallier, C., Paillère-Martinot, M.L., 2006. Left superior temporal gyrus activation during sentence perception negatively correlates with auditory hallucination severity in schizophrenia patients. Schizophrenia Research 87 (1-3), 109–115. http://dx.doi.org/10.1016/j.schres.2006.05.00516828542.

Pu, W., Li, L., Zhang, H., Ouyang, X., Liu, H., Zhao, J., Li, L., Xue, Z., Xu, K., Tang, H., Shan, B., Liu, Z., Wang, F., 2012. Morphological and functional abnormalities of salience network in the early-stage of paranoid schizophrenia. Schizophrenia Research 141 (1), 15–21. http://dx.doi.org/10.1016/j.schres.2012.07.01722910405.

Rolls, E.T., 2012. Glutamate, obsessive–compulsive disorder, schizophrenia, and the stability of cortical attractor neuronal networks. Pharmacology, Biochemistry, and Behavior 100 (4), 736–751. http://dx.doi.org/10.1016/j.pbb.2011.06.01721704646.

Rolls, E.T., 2014a. Emotion and Decision-Making Explained. Oxford University Press, Oxford.

Rolls, E.T., 2013. Limbic systems for emotion and for memory, but no single limbic system. Cortex; a Journal Devoted to the Study of the Nervous System and Behavior. http://dx.doi.org/10.1016/j.cortex.2013.1012.100524439664.

Rolls, E.T., Loh, M., Deco, G., Winterer, G., 2008. Computational models of schizophrenia and dopamine modulation in the prefrontal cortex. Nature Reviews. Neuroscience 9 (9), 696–709. http://dx.doi.org/10.1038/nrn246218714326.

Rosenheck, R., Dunn, L., Peszke, M., Cramer, J., Xu, W., Thomas, J., Charney, D., 1999. Impact of clozapine on negative symptoms and on the deficit syndrome in refractory schizophrenia. Department of Veterans Affairs Cooperative Study Group on Clozapine in Refractory Schizophrenia. American Journal of Psychiatry 156 (1), 88–939892302.

Shinn, A.K., Baker, J.T., Cohen, B.M., Ongür, D., 2013. Functional connectivity of left Heschl's gyrus in vulnerability to auditory hallucinations in schizophrenia. Schizophrenia Research 143 (2-3), 260–268. http://dx.doi.org/10.1016/j.schres.2012.11.03723287311.

Smith, K., 2012. Neuroscience: idle minds. Nature 489 (7416), 356–358. http://dx.doi.org/10.1038/489356a22996531.

Smith, R.C., Chua, J.W., Lipetsker, B., Bhattacharyya, A., 1996. Efficacy of risperidone in reducing positive and negative symptoms in medication-refractory schizophrenia: an open prospective study. Journal of Clinical Psychiatry 57 (10), 460–466. http://dx.doi.org/10.4088/JCP.v57n10048909332.

Stephan, K.E., Friston, K.J., Frith, C.D., 2009. Disconnection in schizophrenia: from abnormal synaptic plasticity to failures of self-monitoring. Schizophrenia Bulletin 35 (3), 509–527. http://dx.doi.org/10.1093/schbul/sbn17619155345.

Tan, H.Y., Sust, S., Buckholtz, J.W., Mattay, V.S., Meyer-Lindenberg, A., Egan, M.F., Weinberger, D.R., Callicott, J.H., 2006. Dysfunctional prefrontal regional specialization and compensation in schizophrenia. American Journal of Psychiatry 163 (11), 1969–1977. http://dx.doi.org/10.1176/appi.ajp.163.11.196917074949.

Tao, H., Guo, S., Ge, T., Kendrick, K.M., Xue, Z., Liu, Z., Feng, J., 2013. Depression uncouples brain hate circuit. Molecular Psychiatry 18 (1), 101–111. http://dx.doi.org/10.1038/mp.2011.12721968929.

Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N., Mazoyer, B., Joliot, M., 2002. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. Neuroimage 15 (1), 273–289. http://dx.doi.org/10.1006/nimg.2001.097811771995.

Van Buuren, M., Gladwin, T.E., Zandbelt, B.B., Kahn, R.S., Vink, M., 2010. Reduced functional coupling in the default-mode network during self-referential processing. Human Brain Mapping 31 (8), 1117–1127. http://dx.doi.org/10.1002/hbm.2092020108218.

Vogt, B.A., 2009. Cingulate Neurobiology and Disease. Oxford University Press, Oxford.

Warn, D.E., Thompson, S.G., Spiegelhalter, D.J., 2002. Bayesian random effects meta-analysis of trials with binary outcomes: methods for the absolute risk difference and relative risk scales. Statistics in Medicine 21 (11), 1601–1623. http://dx.doi.org/10.1002/sim.118912111922.

Whitfield-Gabrieli, S., Thermenos, H.W., Milanovic, S., Tsuang, M.T., Faraone, S.V., McCarley, R.W., Shenton, M.E., Green, A.I., Nieto-Castanon, A., LaViolette, P., 2009. Hyperactivity and hyperconnectivity of the default network in schizophrenia and in first-degree relatives of persons with schizophrenia. Proceedings of the National Academy of Sciences of the United States of America 106 (4), 1279–1284. http://dx.doi.org/10.1073/pnas.0809141106191645 77.

Yang, G.J., Murray, J.D., Repovs, G., Cole, M.W., Savic, A., Glasser, M.F., Pittenger, C., Krystal, J.H., Wang, X.J., Pearlson, G.D., Glahn, D.C., Anticevic, A., 2014. Altered global brain signal in schizophrenia. Proceedings of the National Academy of Sciences of the United States of America 111 (20), 7438–7443. http://dx.doi.org/10.1073/pnas.1405289111247996 82.

Zhou, Y., Liang, M., Tian, L., Wang, K., Hao, Y., Liu, H., Liu, Z., Jiang, T., 2007. Functional disintegration in paranoid schizophrenia using resting-state fMRI. Schizophrenia Research 97 (1-3), 194–205. http://dx.doi.org/10.1016/j.schres.2007.05.02917628434.